48 (amended). A recombinant protein encoded by a polynucleotide which comprises two DNA subsequences, one of said subsequences encoding a soluble fragment of an insoluble protein capable of binding tumor necrosis factor and having an apparent molecular weight of about 55 kilodaltons on a nonreducing SDS-polyacrylamide gel, which soluble fragment is capable of binding human tumor necrosis factor, and the other of said subsequences encoding all of the domains of the constant region of the heavy chain of a human immunoglobulin other than the first domain of said constant region.

REMARKS

Claims 44-55 are pending. Claim 55 has been withdrawn pending filing of a divisional application. Claim 48 has been amended and claim 49 has been cancelled without prejudice. Consequently, claims 44-48 and 50-54 are under consideration.

Claim 48 has been amended to introduce subject matter of dependent claim 49, and claim 49 has been cancelled to avoid redundancy after such amendment. No issue of new matter is raised.

In response to the restriction of the claims into the inventions of Group I and Group II, the election of the invention of Group I, claims 44-54, is hereby affirmed.

Applicants note that formal drawings will be required, and will supply formal drawings at such time as the subject application is allowed.

35 U.S.C. §102 rejection:

Claims 44-47 have been rejected under 35 U.S.C. §102(b) over Stauber et al. (JBC 263:19098). It is alleged that Stauber discloses a homogenous, soluble tumor necrosis factor (TNF) receptor which is the same as the claimed homogenous insoluble TNF binding protein. This rejection is respectfully traversed. Claims 44-47 are directed to a homogenous protein having an apparent molecular weight of about 55 kilodaltons, which binds human tumor necrosis factor (TNF). Stauber does not disclose such a protein.

As is well settled, a §102 rejection must be based on a reference which describes every element of the claimed invention. Stauber does not disclose a homogenous TNF-binding protein, nor does Stauber disclose a 55 kilodalton protein. Therefore, Stauber does not disclose the claimed homogenous 55 kilodalton TNF-binding protein.

Stauber does not disclose a homogenous TNF receptor. The preparation of Figure 6 demonstrates Stauber's highest level of purification (after immunoaffinity chromatography and SDS-PAGE), but is not a homogenous preparation of TNF-R. The silver-stained gel in Figure 6 shows a mixture of different bands, and not a homogenous protein. Stauber states with regard to the silver-stained gel, that the reduced lane contains other bands

> "...when the sample was run under reducing conditions, the majority of silver-stained protein appeared at 85 kDa. Some additional material remains in the 100,000-dalton region and most likely represents a contaminant which has copurified with the receptor-ligand complex. There are also some minor bands staining between the 42- and 97-kDa markers." (paragraph joining pages 19101-19102).

Thus Stauber's most purified preparation does not provide homogenous receptor.

According to Stauber, the gel of Figure 6 corresponds to a 165,000-fold purification of TNF receptor. However, this does not mean that the preparation is homogenous, at best it means that the preparation is 165,000 purer in TNF receptor than the starting material, a cell lysate. Since the gel of Figure 6 contains multiple bands, this demonstrates that Stauber does not have a homogenous preparation, no matter what the extent of purification. Stauber notes that his 165,000-fold purification corresponds to only 20% purity, which does not approach homogeneity. And this does not even represent purity of the TNF receptor, but of a complex of TNF receptor covalently linked to TNF-a, which is not the same chemical entity as a free receptor.

> "The combined purification by both immunoaffinity chromatography and preparative SDS-PAGE was approximately 165,000-fold. The overall yield for these two steps was estimated to be about 34 ng of receptor from approximately 1.5 x 10^{10} U937 cells based on the content of ^{125}I labelled TNF-a in the product. The cross-linked receptor is approximately 20% pure at this point." (page 1903, column 1, paragraph 2).

Clearly the Stauber reference does not disclose homogenous TNF. The reference itself states that it is an impure preparation. Therefore, Stauber does not provide basis for a §102 rejection of a homogenous protein as claimed.

Furthermore, Stauber does not disclose a 55 kilodalton protein, even less a homogenous 55 kilodalton protein. The closest this reference comes is disclosing impurities which fall in a molecular weight range encompassing 55 kilodaltons. This is far from a disclosure of a 55 kilodalton protein, even less a homogenous 55 kilodalton protein. Specifically, the preparation of Figure 5 has bands between 45 and 66 kilodaltons. These bands are disclosed to be contaminants (see page 19101, column 1, lines 4-5). In addition, this preparation contains complexes of TNF receptor covalently bound to ligand, not TNF receptor. The preparation of Figure 6 also contains bands between the 42 and 97 kilodalton markers, and these are also disclosed as contaminants or

proteolytic fragments (page 19102, column 1, lines 4-6). These impurities do not represent a purified 55 kilodalton protein, which would be present in an isolated 55 kilodalton band, even less a homogenous 55 kilodalton protein. In addition, these presence of these impurities emphasizes that the Stauber preparations, even of Figure 6, are not homogenous.[1] Thus, Stauber discloses neither homogenous TNF receptor or a 55 kilodalton protein, and does not anticipate the claimed homogenous 55 kilodalton TNF-binding protein.

Based on the foregoing, applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 44-47 under 35 U.S.C. §102(b).

35 U.S.C. §103 rejection:

Claims 48-54 have been rejected under 35 U.S.C. §103 over Wallach et al. (EP 308 378) in view of Capon et al. (WO 89/02922). This rejection is respectfully traversed

Claims 48-53 are directed to a fusion protein including parts of the 55 kilodalton protein and an immunoglobulin. Claim 54 is directed to an antibody to the 55 kD protein. The claims are rejected over Wallach as allegedly disclosing a 55 kilodalton TNF-binding in view of Capon, which discloses certain immunoglobulin fusion proteins. With regard to the protein claims, Wallach does not disclose or suggest a 55 kilodalton TNF-binding protein, even less an insoluble one. Therefore, no 55 kD TNF-binding protein is available to combine with Capon.

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[1] Activity at 65 kilodaltons is disclosed in the gel filtration analysis of Figure 7b, which shows that the TNFa binding activity in a cell extract peaks at around 65 kilodaltons. This can hardly be considered to disclose any specific molecular weight protein, whether 65 kilodaltons or otherwise. The gel filtration was performed on a cell extract not otherwise purified, and the activity was spread over a broad range of molecular weights (page 19102, column 1, lines 31-35).

The Examiner states that Wallach discloses a 40-80 kD protein. However, this molecular weight pertains to an impure intermediate which has not yet been purified. This is not the molecular weight of the purified Wallach protein. As stated by Wallach

> "The TNF Inhibitory Protein of the invention may be found in human urine. When crude preparations thereof derived from human urine concentrate were chromatographed on Ultrogel ACA 44 gel filtration column, it showed an apparent molecular weight of 40-80 Kda. The substantially purified protein, which is substantially free of proteinaceous impurities, has a molecular weight of about 26-28 Kda..."(page 3 lines 46-50, emphasis added)

As this demonstrates, the preliminary and impure preparation of Wallach has fractions in the molecular weight range of 40-80 kD. However the Wallach protein is a protein having a molecular weight of 26-28 kD. Wallach does not disclose a purified protein having a molecular weight of 55 kD.

In contrast, the protein of the claimed fusion protein has a molecular weight of 55 kD when purified. This is the molecular weight of the claimed protein after it has gone through reversed phase HPLC and SDS-PAGE (specification, page 8 lines 20-23 and Examples 5 and 6), which are the same procedures the Wallach protein must go through before it, also, is purified, and determined to have a molecular weight of 26-28 kD.

> "...applying said partially purified ...TNF Inhibitory protein...to..(HPLC) to obtain substantially purified active fractions of the TNF Inhibitory Protein...and...recovering the substantially purified protein...said protein having a molecular weight of about 26-28 kDa on SDS PAGE..."(page 6 lines 45-50)

The claimed protein and the Wallach protein go through different preliminary purification steps, because the Wallach protein is soluble and isolated from urine and the claimed protein is insoluble and isolated from cell membranes. However, both proteins reach their final phase of purification after HPLC and SDS-PAGE, and are clearly different proteins, having different molecular weights.

Since Wallach does not disclose a purified 55 kD protein, there is no suggestion in Wallach that such a protein exists. Therefore, Wallach provides no motivation or guidance to obtain a 55 kD protein, even less the claimed fusion protein requiring the 55 kD protein as one of its components.

Even if Wallach, by disclosing a general theoretical method for isolating a desired protein by using a cDNA probe, disclosed sufficient information to ultimately find a 55 kD protein (which applicants do not concede), that such a 55 kD protein could be found is in no way suggested by Wallach, and would not be obvious to a skilled person. All that Wallach discloses is a 26-28 kD protein, and at most all that Wallach suggests to a skilled person is using recombinant methods in an attempt to find a gene encoding the Wallach protein. There is no suggestion that a larger protein exists, even less that such a protein should be looked for, and no guidance for such a protein being found.

In this regard, attention is directed to In re Kratz, 201 USPQ 71 (CCPA 1979). In this case, the CCPA reversed a §103 rejection of a composition containing a purified strawberry flavor (2M2PA), found naturally in strawberries. The CCPA stated that, even if 2M2PA were a known ingredient of strawberries, and although the process of determining flavor compounds was well-known, since was no disclosure in the art of using 2M2PA as a strawberry flavor the rejection was improper.

> "While recognizing that obviousness does not require complete predictability, (citations omitted), we would consider it necessary, even once 2M2PA is known, that the prior art itself provide *some* foreseeability or predictability that the compound is a significant strawberry flavor ingredient. We have previously rejected the argument that undirected skill of one in the pertinent art is an adequate substitute for statutory prior art (citations omitted). We emphasize that there is a difference between somehow substituting skill in the art for statutory prior art, as the PTO attempts here, and using that skill to interpret prior art (citations omitted). (at 76)

Here, as in Kratz, the art does not disclose the nature of the claimed invention (a 55 kD protein), and in the absence of such suggestion, there is no basis for a §103 rejection. As shown in Kratz, even if skill in the art is available for obtaining the claimed invention, this is no basis for rejection in the absence of any suggestion of the claimed invention itself, a fusion protein comprised of a 55 kD protein. In accordance with Kratz, a §103 rejection is improper.

Not only does Wallach fail to suggest a 55 kD protein, Wallach also does not suggest an insoluble protein. The Wallach protein is obtained from urine (see page 6 line 40 "... the substantially purified protein of the invention is produced by a process which comprises ... recovering ... protein ... from ... urine.") because it is soluble - if insoluble it would not be free in urine. A soluble protein obtained from urine is the only actual purified protein that Wallach discloses. In contrast, the claimed protein is insoluble and must be isolated from cell membranes. Since Wallach does not disclose a purified insoluble protein, there is no suggestion in Wallach that such a protein exists. Thus Wallach provides no motivation or guidance to obtain an insoluble protein. Exactly as shown above, there is no suggestion that an insoluble protein could either be sought after or obtained.

In the same way that Wallach does not disclose or suggest the 55 kD insoluble protein of the claimed fusion protein, Wallach equally does not suggest fragments of the claimed protein. As has been shown, Wallach does not suggest to or motivate a skilled person to obtain a 55 kD protein. Therefore, even less does Wallach suggest or motivate a skilled person to obtain fragments of such a 55 kD protein.

In the absence of any disclosure or suggestion of the claimed insoluble 55 kD protein, the antibody of claim 54 directed against the claimed protein is also not suggested. If there is no protein available, even less is it possible to obtain an antibody to that protein.

Wallach is cited to disclose a 55 kD TNF-binding protein as claimed. In addition to not disclosing such a protein, Wallach also does not disclose fusion proteins. Capon is cited to disclose immunoglobulin fusion proteins. Since Capon does not disclose any TNF-binding proteins, and Wallach does not disclose a 55 kD insoluble protein as claimed, even in combination there is no suggestion of the claimed fusion protein. These references individually or in combination do not suggest the claimed fusion proteins.

However, even if the claimed protein were disclosed in the art, there is no basis to combine such a protein with the fusion proteins of Capon. Capon discloses specific fusion proteins made up only of those members of the immunoglobulin superfamily which are not highly polymorphic (page 6 lines 18-21). The claimed protein is not a member of the immunoglobulin superfamily, not even a highly polymorphic member. It is well settled that for a combination rejection there must be some teaching or suggestion in the references themselves that suggest the combination. (In re Fine, 5 USPQ2d 1596, 1598 (CAFC 1988; In re Vaeck, 20 USPQ2d 1438 (CAFC 1991)). Capon does not suggest immunoglobulin fusion proteins made with proteins which are not members of the

immunoglobulin superfamily. Thus there is no suggestion in the art to make the claimed fusion proteins.

In addition, even in combination the cited references do not suggest the unexpected property of the claimed fusion protein to bind to TNF with higher affinity than recombinant TNF receptor itself. This is demonstrated in Figure 2 of Loetscher et al., J. Bio. Chem. 266:18324 (September 1991) attached hereto as Exhibit 1. Attention is directed to page 18326, column 2, 1st paragraph, which states

> "The apparent affinity of the bivalent TNFRß-ht3 fusion protein for TNFa and TNFß was found to be significantly higher than the affinity of ... monovalent rsTNFRß (Fig. 2)."

As can be seen from Figure 2, the dissociation constants (Kd) obtained for the fusion protein are 0.10 nm and 0.12 nm, whereas the dissociation constants obtained for recombinant TNF receptor are 0.38 nm, 0.52 nm, 0.34 nm, and 1.6 nm. A lower dissociation constant indicates stronger binding affinity, since kD reflects the concentration at which binding will occur. Thus, the claimed TNF receptor fusion protein has the unexpected property of superior binding to TNF over the "unfused" TNF receptor. As is well-settled, unexpected results overcome a §103 rejection.

Based on the foregoing, applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 54-57 and 88-89 under 35 U.S.C. §103. .

Disclosure of an English abstract for non-English patent publication EP 464 533 has been requested. However, applicants have already disclosed the requested English abstract. EP 464 533

was accompanied by English abstract 92-009794/02 when disclosed in the May 19, 1995 Information Disclosure Statement. In addition, the priority date of this application predates the publication of EP 464 533.

A Petition for a one month extension of time is enclosed herewith.

Applicants respectfully solicit allowance of the subject application.

Respectfully submitted,

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Your Amendment

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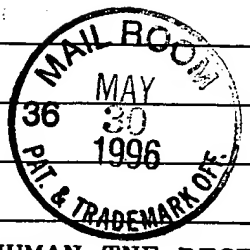
Brockhaus, et al. - HUMAN TNF RECEPTOR



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